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SUPPLEMENTAL MATERIAL FOR:

E. coli DNA Replication in the Absence of Free Clamps

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Protein Labeling

and SSB were labeled using an amine-reactive NHS-ester–Cy3-Cy5 heterodimer, prepared as described in Conley et al (2008). Protein stocks were dialyzed into PBS (pH 7.6) in order to remove Tris amine groups and have optimum pH for N-terminal labeling (Etson et al, 2010). Dye was mixed with at a 10:1 molar ratio and SSB at 6:1, incubated for 2 h at room temperature, and the labeled protein removed from free dye by exhaustive buffer exchange in a concentrator (Vivaspin 500, GE). The degree of labeling was determined to be 2 Cy5:1 $_2$ and 1.1 Cy5:1 SSB_4 as measured with UV-Vis absorbance (A_{280} corrected for A_{dye}) and comparative SDS-PAGE (Alexa555- and Cy3-Maleimide- were produced with similar protocols).

Labeled Activity

Single-molecule tethered-bead leading-strand synthesis experiments (Tanner et al, 2008) were conducted with Cy- , and we measured a processivity of 12.4 ± 2.1 kb (vs. 10.5 ± 0.9 kb unlabeled) and rate of 442 ± 48 bp s^{-1} (417 ± 8 bp s^{-1} unlabeled). In single-molecule rolling-circle experiments (Tanner et al, *Nucleic Acids Res*, 2009) we measured a product length of 78 ± 8.6 kb (85.3 ± 6.1 kb unlabeled). No decrease in efficiency was observed in either experiment. Bulk-phase rolling-circle experiments (1 nM M13 substrate, 10 mM DTT, 40 μM dNTP, 200 μM rNTP, 20 nM $_3$ ′ , 30 nM , 30 nM $_2$, 30 nM $DnaB_6$, 180 nM DnaC, 250 nM SSB_4, 300 nM DnaG, 20 nM PriA, 40 nM PriB, 480 nM PriB, 320 nM PriC; incubated 20 min at 37 °C quenched with 100 mM EDTA) were conducted using variously labeled and analyzed using 0.5% alkaline agarose gel electrophoresis (Supplemental Figure 3). None of the labeled displayed decreased synthesis or aberrant Okazaki fragment length as compared to unlabeled.

DigUTP Incorporation

Reactions were performed as above but without DnaG where indicated and increasing amounts of digUTP (Roche) as indicated. Total [UTP] was kept constant at 200 μM, and ATP, CTP, GTP were added at 200 μM. Replication products were purified from free digUTP by Sephadex G-25 gel filtration (PD Spintrap G-25, GE LifeSciences) and spotted on positively charged nylon membranes (Roche). Digoxigenin was detected by alkaline phosphatase/NBT-BCIP immunodetection (DIG Nucleic Acid Detection Kit, Roche). Dot blot is shown in Supplemental Figure 5.

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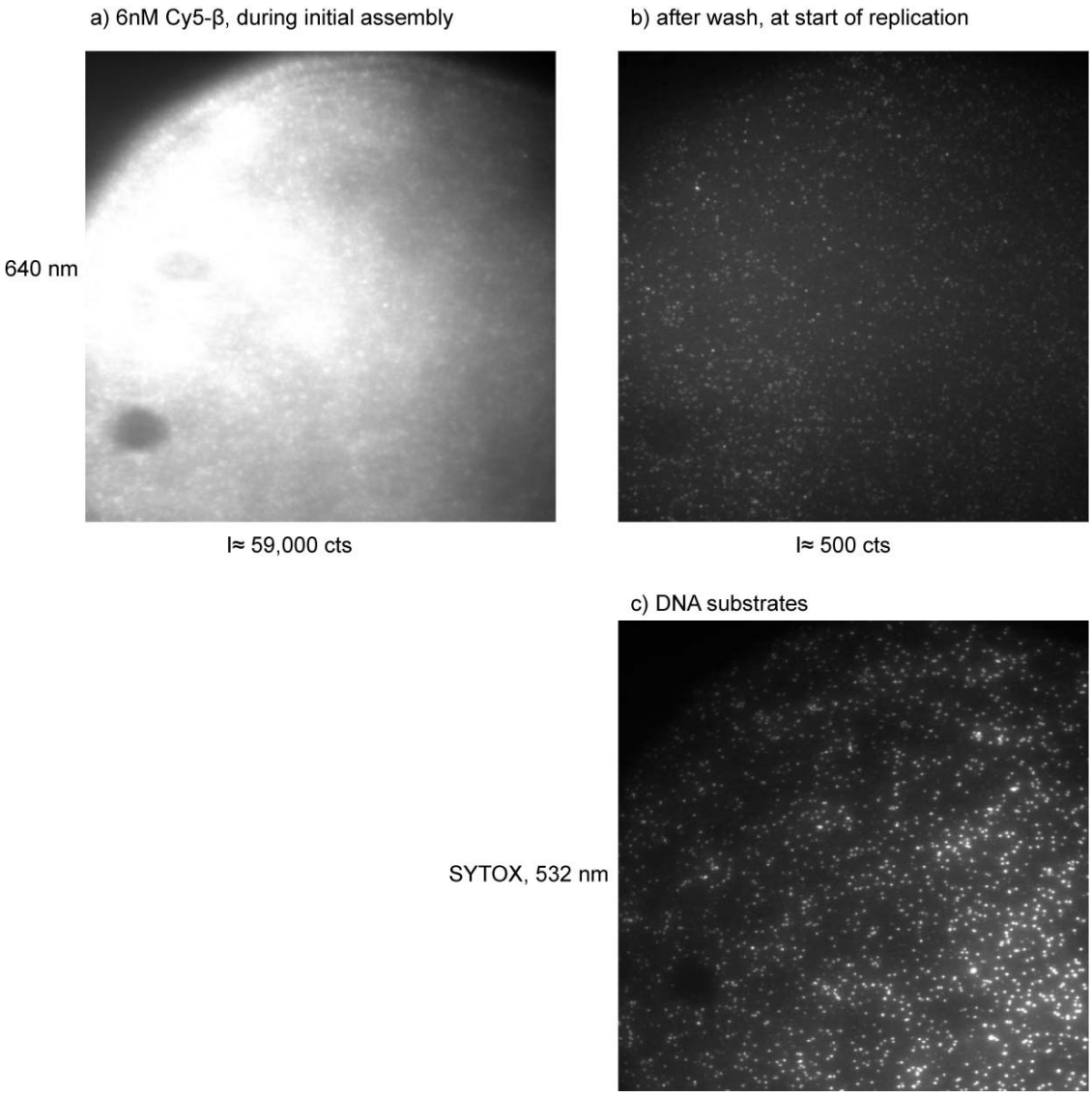
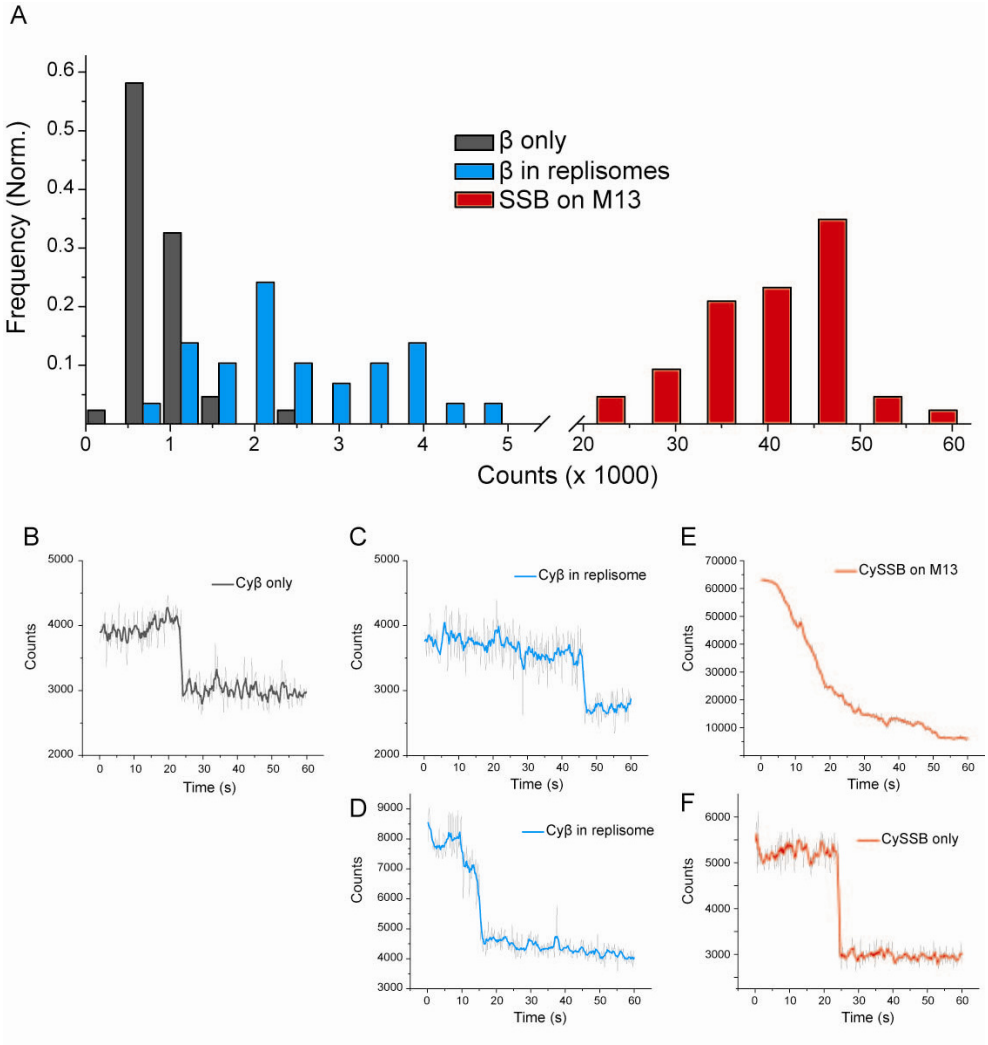
SUPPLEMENTAL FIGURE 1



a) Image of initial solution (Solution 1+2, diluted in Wash) as it is introduced into the flow chamber.
b) Image after wash step, as replication solution (Solution 3) is introduced into the flow chamber. Numbers represent integrated intensity (full chip) values corrected for the electronic offset of the EMCCD camera (1,485 counts).
c) 532 nm image of DNA substrates. Surface density of DNA is comparable to density of Cy5- shown in b).

SUPPLEMENTAL FIGURE 2



Fluorescence intensity decrease of labeled proteins

a) Intensity decrease histograms, showing Cy- alone (grey), Cy- in replisome spots (blue), and SSB on ssM13 (red). Break in x-axis is to illustrate the dramatic intensity difference between protein-filled DNA and small numbers of in replisomes. Y-axis shows total counts of 3x3 pixel box around replisome spot, and includes electronic offset of the EMCCD (1,485 counts).

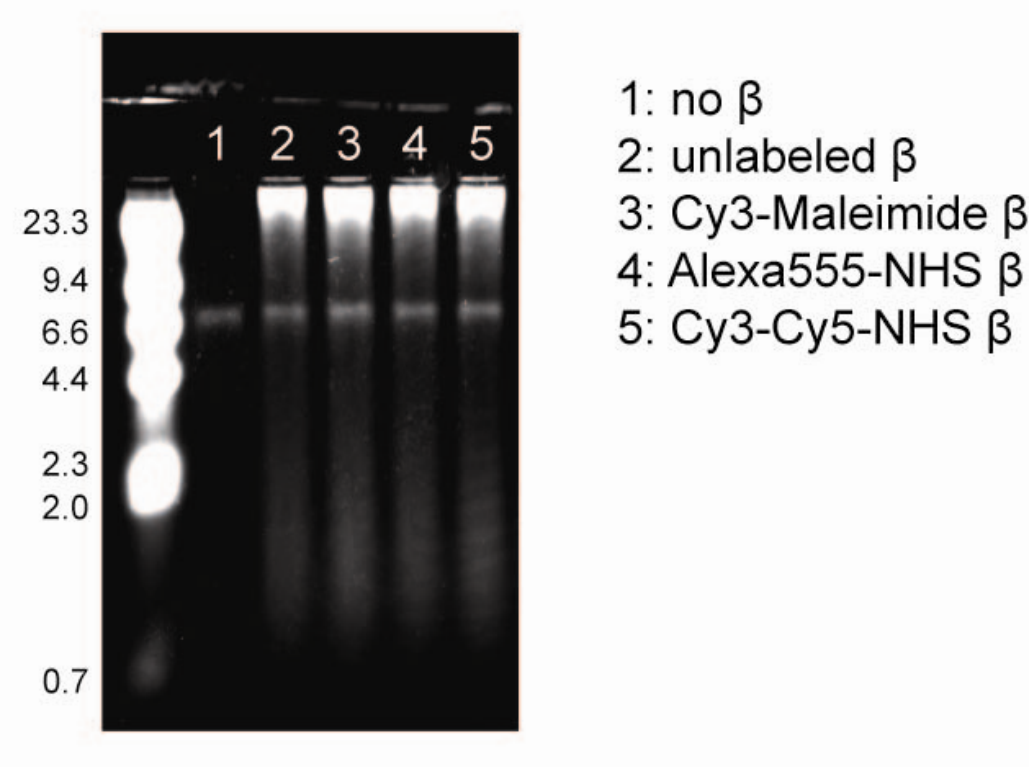
b) Bleaching trajectory of Cy- alone. Colored line indicates 1 s adjacent averaging of the intensity trace, background grey.

c,d) Bleaching trajectories of Cy-B replisome spots, showing single (c) and stepwise (d) bleaching

e) Bleaching of Cy-SSB on ssM13, with exponential bleaching curve due to the high number of fluorescent molecules.

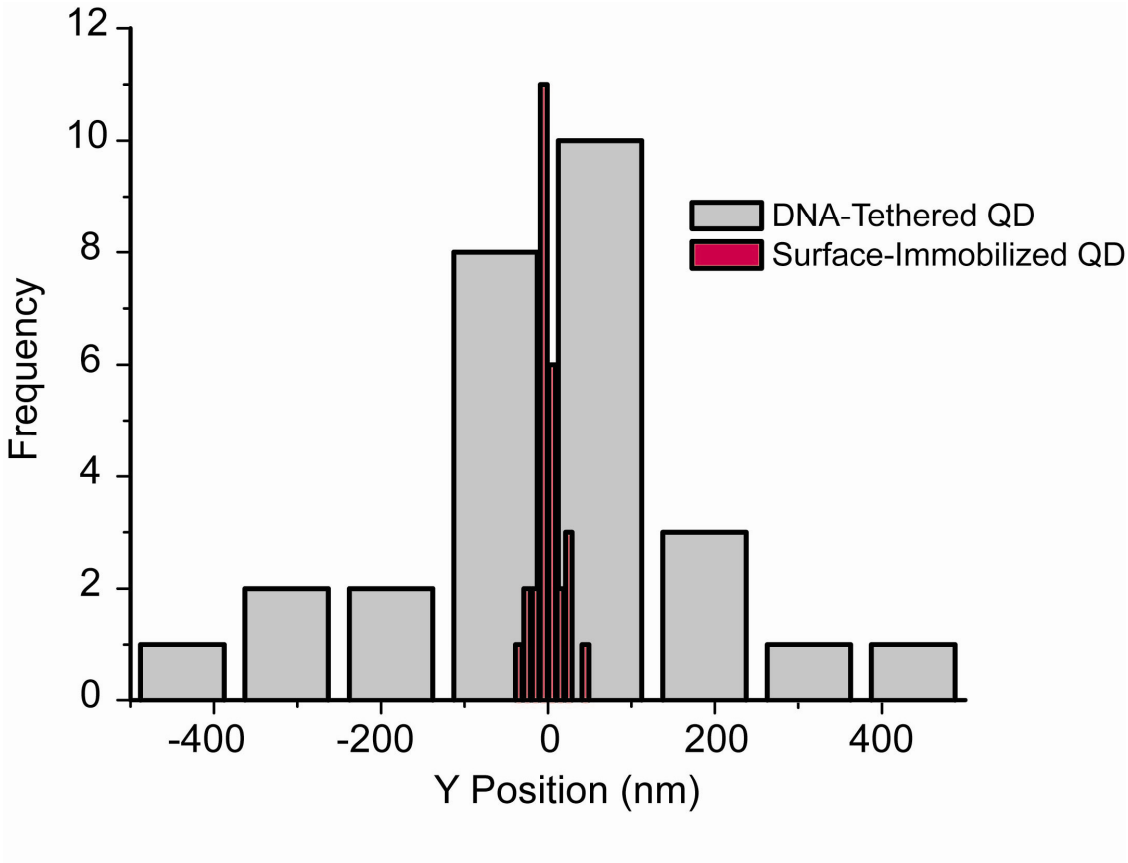
f) Single Cy-SSB without DNA display single bleaching events as with the Cy- , indicating that (e) is indeed large groups of SSB on DNA.

SUPPLEMENTAL FIGURE 3



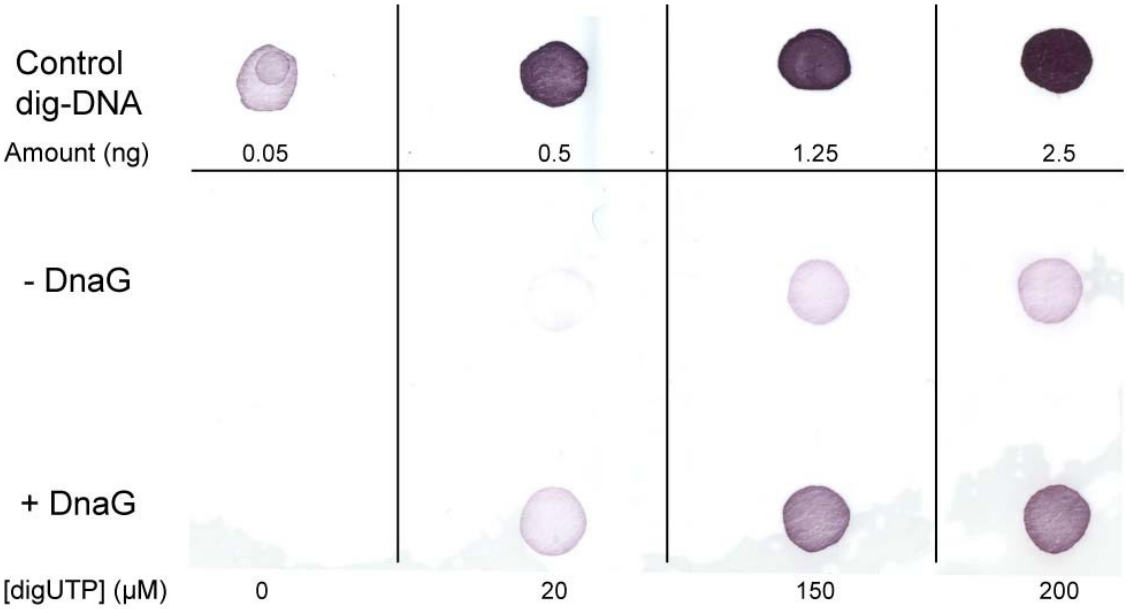
Reactions performed as described above, products separated with 0.5% alkaline agarose gel electrophoresis and stained with SYBR Gold. Ladder is DNA-HindIII Digest (New England Biolabs) and sizes indicated in kb. Long leading-strand product remains near the well, a 7.2 kb band indicates nonreplicated M13 substrate, and broad Okazaki fragment distribution can be seen at 1–2 kb.

SUPPLEMENTAL FIGURE 4



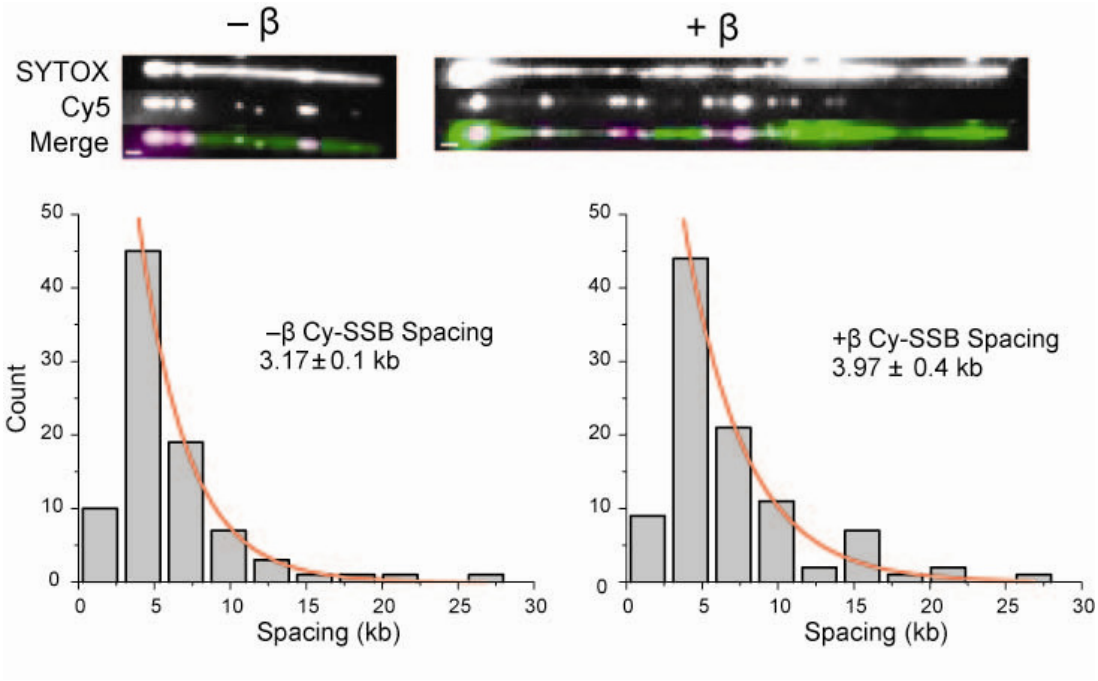
Position histogram of two Quantum Dots, one tethered to DNA at a dig-UTP site, the other stuck to the surface. Positions are fit with Gaussians using tracking software (DiaTrack), which increases spatial resolution beyond the optical diffraction limit. The DNA-tethered QD exhibits dramatic position fluctuations as it is on the fluctuating DNA, while the surface-immobilized QD effectively does not move. This clear difference is used to discriminate against QD on the surface of the flow chamber.

SUPPLEMENTAL FIGURE 5



Reactions and detection were performed as described above. Presence of DnaG resulted in ~5x digoxigenin incorporation over –DnaG digoxigenin background. Amount of digUTP initially in reaction was significantly higher (7.2 μg in 200 μM conditions), showing relatively efficient purification of free digUTP (~20 pg detected –DnaG).

SUPPLEMENTAL FIGURE 6



Images of products replicated with fluorescent SSB. DNA is imaged using SYTOX Orange and SSB with Cy5, and the images merged. Both show patterns of SSB spots along the product, indicating that even without free multiple Okazaki fragments are synthesized. Total number of SSB spots is lower in the – image due to smaller size of the molecule, 55 kb vs. 127 kb + . Scale bars represent 4 kb. Below images are distributions of spacing between Cy-SSB spots in the two conditions (both with 300 nM DnaG). Red line indicates exponential fit and value is fit decay constant ± standard error. The first bin is excluded from fitting due to undersampling; we cannot confidently resolve Quantum Dots closer than ~2–3 (~1.5–2 kb) pixels apart.